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An Investigation of the Reliability of Root and Shoot Growth Inhibition of Germinating Seedlings in Determining Relative Phytotoxicity of Various Chemical Compounds

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AN INVESTIGATION OF THE RELIABILITY OF ROOT AND SHOOT GROWTH INHIBITION OF GERMINATING SEEDLINGS IN DETERMINING RELATIVE PHYTOTOXICITY OF VARIOUS CHEMICAL COMPOUNDS

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INTRODUCTION

Previous to 1940, sodium chlorate, certain boron compounds, and arsenic compounds were established as soil sterilants. Immediately prior to this time, the use of sulphuric acid and substituted phenols had attracted some attention and favor as selective herbicides. When 2,4-D (2,4-dichlorophenoxyacetic acid) became available as an herbicide its spectacular efficiency not only facilitated its wide acceptance but also aroused a new and greater interest in chemical weed control.

In response to the demand for new herbicides to meet the recognized inadequacies of 2,4-D and the older herbicides, many chemicals of possible herbicidal value are now being synthesized and screened by numerous formulators. The procedures of screening vary from simple single plant laboratory tests to extensive greenhouse and field trials. None of these procedures are completely satisfactory in that they were developed to estimate a reaction similar to that exhibited by hormone type growth regulators such as 2,4-D.

This paper reports a laboratory screening test designed to give an adequate evaluation of chemical compounds with respect to their herbicidal potentialities. The basis for this test was derived from earlier work done with systemic growth regulators, and it was recognized that the various compounds to be tested might not all react in a similar manner.

Greenhouse trials with the same chemicals were run similtaneously to check the accuracy and reliability of the laboratory test.

REVIEW OF LITERATURE

After 1931, considerable work was reported concerning the study of plant growth regulators. Zimmerman (17) (1941) investigated the essential structural characteristics of these substances, and made a special study of phenoxyacetic acid and its derivatives. He applied the various compounds as water solutions and as vapors to eleven different species of plants. He noted the importance of the halogen substitutions and reported parachlorophenoxyacetic acid and 2,4-dichlorophenoxyacetic acid to be highly active.

In 1944, several workers independently suggested that synthetic growth regulating substances could be used as herbicides (16). Slade et al (10) confirmed the importance of the chlorine or bromine substitutions on phenoxyacetic acid and also reported on the selectivity of 2,4-D. Blackman (3) compared the effects of 2,4-dichlorophenoxyacetic acid and 2-methyl, 4-chlorophenoxyacetic acid.

While 2,4-D resulted from the development of the early knowledge of the action of a group of hormone-like substances another series of compounds were known to have growth regulating activity. This is the urethane series; members of which as early as 1929 were reported to be growth inhibiting. Of this series, IPC (isopropyl N-phenyl carbamate) was reported to be the most active.

The unveiling of the potentialities of these herbicides stimulated the screening of additional chemical compounds. Tests were conducted in an effort to find compounds with specific herbicidal properties or with properties superior to those of the herbicides now available. In 1944, Thompson (16) reported on eleven hundred organic compounds especially prepared and screened in an effort to find a compound with more activity than 2,4-D. In referring to fourteen papers previously reporting on the evaluation of plant growth substances, he concluded that a new method of testing must be devised for his project. He reported that none of the earlier tests would give a quantitative expression of the herbicidal qualities of a large group of substances being tested at different times. For his purposes it was considered satisfactory to express length and weight measurements of plant growth increment following application of a test herbicide as a percentage of the increment produced following treatment by a reference material, 2,4-D. In all, he used three distinct tests. The first was the kidney bean single droplet water test. One drop of an aqueous solution of the herbicidal meterial was placed on one of the primary leaves. After a fixed period of time, growth measurements were taken, and these were expressed as a percent of a comparable growth increment as modified by 2,4-D.

The second test was essentially similar except that the test chemicals were suspended in oils for application. His corn germination test was the third. Corn seed was placed on filter paper in six inch petri dishes, and germinated at 27° C. Twenty millimetres of an aqueous solution of the test herbicide at a concentration of 10 ppm were added to each dish. After 96 hours, the length of the primary root was determined and expressed as a percentage of the length of the roots of seedlings treated with comparable concentrations of 2,4-D. Since conditions of growth and treatment could be rigidly controlled using this technique, the germination test was considered most desirable. Compounds, which were highly active, could be separated with this test. Complete agreement was not found between data of the three tests, and in some instances growth of treated seedlings exceeded that of the untreated checks.

In support of the accuracy of Thompson's corn germination test Swanson (12) used a modification of it as a bio-assay test, and established a reference curve for very low concentrations of 2,4-D in aqueous extracts. Unknown concentrations of solutions of 2,4-D between .001 and 2 ppm could be accurately determined; less accuracy was obtained with concentration from 2-10 ppm. By dilution techniques concentrations within, or above, this latter range were accurately assayed.

Ready and Grant (9) in an attempt to extend the range of Swanson's bio-assay test examined the sensitivity of species from the families Leguminosae, Compositae, Gramineae Cucurbitaceae, Liliaceae, Malvaceae, and Polygonaceae. The common cucumber, Cucumis sativa, was found to be very sensitive to 2,4-D. By taking measurements of both the primary root and the shoot, two growth curves were established. The curve for the former exhibited high statistical significance for inhibition at concentrations of 2,4-D between .01 and .1 ppm. Between .05 and 5 ppm a similar relationship was established for inhibition of shoot lengths. This work emphasized the reliability of data derived from measurements of the primary root and shoot growth of seedlings as modified by the action of 2,4-D and related compounds.

Once the potency of 2,4-D was established workers began investigating the possibilities of its use as a preemergence treatment. In the course of these studies numerous facts became known regarding the effects of herbicides on seeds and seedlings. Allard et al (1) treated the seeds and seedlings of twenty-two species with 2,4-D, and then grew them in moist chambers or in soil. In some cases seeds were delayed in germination rather than killed and thus the percentage of germination was indicated to be an unreliable criteria of toxicity. Fresh weight and length increment determinations proved to be more dependable. In his early work, a lack of selectivity in the action of 2,4-D between the seedlings of test species was reported. In a later paper, (2), however, considerable information regarding the differential reaction of seedlings to 2,4-D and IPC was established. Seedlings of oats, wheat, corn, barley, and non-flooded rice were severely stunted by treatment with IPC while 2,4-D at similar dosages did not affect these species.

Work by Hamner (5) helps to clarify the reactions of seedlings to treatment with 2,4-D. He studied the possibilities of using this herbicide as a soil treatment to kill weed seeds therein. In addition to treating soil and manure containing weed seeds with concentrations of 0.1, 1, 10, 100 and 1000 ppm of 2,4-D, seeds were soaked in solutions of 1, 10, 100 ppm of the herbicide, and later germinated in moist sand. He reported that concentrations as low as 1 ppm of 2,4-D proved to be as effective in controlling seedlings of certain weed species as applications of 1000 ppm of 2,4-D to top growth in spray solutions.

While in many reported cases grass seedlings were killed they were generally more tolerant of 2,4-D than seedlings of the dicotyledonous plants. Higher concentrations of the herbicide were found necessary to kill seedlings of the monocots. When these facts are considered, it is evident that selectivity may be determined when seedlings are treated if an adequate range of concentrations is used. Mitchell (7) substantiated this while working on a similar project. In a study of the germination of seeds in soil containing 2,4-D he was able to show that selectivity is apparent when seedlings are treated. He reported that soil concentrations of 2,4-D which resulted in a 90% reduction in emergence of mustard plants did not inhibit the germination and growth of barley.

Ennis (4) treated the seedlings of a number of species in evaluating the effects of IPC and found that a lack of root and shoot elongation was the characteristic response. Inhibition of root and shoot length of all monocotyledonous seedlings was accompanied by concurrent swelling of these parts. When thirty-nine dicotyledonous species were treated at the germination stage only fifteen were affected by IPC.

Swanson (13) compared the effects of the sodium salt of 2,4-D and the ammonium salt of TCA (trichloroacetic acid) on germinating sugar beet seed. While 2,4-D caused marked inhibition at the lowest Concen rate (25 ppm) similar inhibition was not caused by even the highest Concen rate of TCA (4000 ppm). TCA caused a stimulation of sugar beet seedlings at lower concentrations. This stimulation was as high as 108.8% at 250 ppm.

Tishler et al (15) used a germination procedure to compare a new group of compounds, the 3,6-endoxohydrophthalic acid and its salts. The free acid of this compound was shown to cause marked inhibition of root and shoot growth of both cucumbers and rice seedlings. King (6) reporting on experimental herbicides states that E.H.1 (Sodium 2,4-dichlorophenyl "cellosolve" sulfate) in sterilized cultures showed little toxicity to plant growth; however, when soil was added to the culture the material was inhibitory to germination. In field tests many horticultural crops proved to be tolerant of E.H.1. E.H.2 (dichloral urea) is reported to have properties similar to those of the trichloroacetates because they exhibit an inhibitory effect upon grass seedlings.

METHODS AND MATERIALS

Fundamentally this screening test consists of growing seeds of three selected species in three inch petri dishes containing different concentrations of the herbicide being evaluated. Evaluations of herbicidal action were made by expressing the inhibition to the primary root and shoot length. In each case these were compared to an untreated check.

Before the actual screening could be undertaken two questions had to be answered.

(1) What is the best technique for germination?

(2) What species should be used?

Good germination can be obtained when seeds are grown on blotters or filter paper in covered petri dishes as was done by previous workers (16), (12), (13), (9). When solutions of unstable compounds, such as are often encountered in this work, are added to the dishes the chemical materials may be precipitated in which case the young seedlings may not come in contact with the herbicidal material when the seed is grown on top of blotting paper. Efforts to obtain even germination without blotters proved unsuccessful, due to the fact that the convex curvature of the bottoms of petri dishes gave variable contact of the seed with the solutions of the herbicides. Good germination and subsequent growth was obtained when seeds were spread evenly over the bottom of the dish and a moistened blotter placed on top of the seeds. Using this procedure both roots and shoots of the seedlings made better contact with the material irrespective of the tendency of the shoots to grow upward. Also, the fact that this method limits root and shoot growth to one plane further facilitated length determination. Under this arrangement seeds were kept uniformly moist, and the primary roots and shoots were kept in contact with the herbicidal materials irrespective of any tendency of the compound to precipitate out. Blotters cut to fit the bottoms of the dishes were dipped in prepared concentrations of the test herbicide and placed over the seeds quite readily. By allowing excess solution to drain from the moistened blotters there was little variability in the amount of chemical in each dish. Before placing the cover on the petri dish, the top side of the blotter was labelled with an indelible pencil. Periodic inspection was made by viewing the seed or seedlings through the bottom of the dish without disturbing the blotter or seed and without opening the dish.

In testing for the optimum temperature for germination and growth, there was a limited choice. Since the seeds were germinated in the Seed Testing Laboratory at South Dakota State College it was necessary to conduct this preliminary test at the temperatures at which the germinators were being maintained. These temperatures were alternating 20°-30°C., 20°C., and 17°C. The data of Table 1 indicate that root and shoot growth of the three test species was greater under conditions of the alternating 20°-30°C. than under either 17°C. or 20°C. Percent germination of all three species was likewise satisfactory at the alternating temperature. At

17°C. the foxtail failed to germinate. On the basis of this trial the alternating temperature of 20-30°C. was selected for the germination of seeds throughout these tests. Under these condtions seeds and seedlings were exposed to a temperature of 20°C. for twelve hours and 30°C. for twelve hours each day.

Table 1. Mean root and shoot length in millimetres and percent germination of seeds of three species under different temperatures in laboratory germinators for a 96 hour period.

	20-30°C. 1/			20°C.			17°C		
	% Germ.	Length Root	Length Shoot	% Germ.	Length Root	Length Shoot	% Germ.	Length Root	Length Shoot
Wheat	94	53.3	7.7	92	27.4	4.4	88	13.5	2.4
Alfalfa	67	22.4	14.5	56	18.5	7.4	47	10.3	3.4
Setoria	67	26.4	26.2	72	60.3	31.1	0	0.0	0.0

1/ Alternating temperature of 20°C. 12 hours; 30°C. 12 hours each day.

A preliminary experiment was then conducted to determine the best species of seed to use in these tests. The primary purpose of using more than one species was to establish a basis for detecting possible selectivity of action of the herbicides. A number of selected species were germinated in a standard incubator under an alternating temperature of 20-30°C. (Table 2). These were alfalfa (*Medicago sativa*), sweet clover (*Melilotus alba*), red clover (*Trifolium pratense*), common spring wheat (*Triticum vulgare*), green foxtail (*Setaria viridis*), yellow foxtail (*Setaria lutescens*), barnyard grass (*Echinohloa crusgalli*), quackgrass (*Agropyron repens*), wild oats (*Avena fatua*).

Table 2. The percent of germination of seed of nine species after ninety-six hours in a laboratory germinator operating at an alternating temperature of 20°-30°C. Also observations on uniformity of germination and growth after 96 hours, 120 hours, and 144 hours in the germination chamber.

	% germ. 96 hours	Notes on germination and growth		
		96 hours	120 hours	144 hours
Medicago sativa	82	growth uniform	growth uniform	growth uniform
Melilotus alba	79	growth uniform	growth uniform	growth uniform
Trifolium pratense	78	growth uniform	growth uniform	growth uniform
Setaria lutescens	33	growth irregular	growth irregular	growth irregular
Setaria viridis	58	growth uniform	growth uniform	root tip turning brown
Agropyron repens	38	germination just beginning	little growth	growth irregular
Avena fatua	92	shoot growth uneven	growth irregular	growth forming a mat
Echinochloa crusgalli	39	growth retarded	growth irregular	growth irregular
Triticum vulgare	96	growth uniform	growth forming a mat	dense mass of roots

In Table 2 it will be noted that seeds of all crop plants, wheat, red clover, sweet clover, and alfalfa, showed a relatively high rate of germination. Growth was initiated and proceeded uniformly, and all species were in a comparable stage of growth throughout the period. From this group wheat was chosen because it is a monocotyledonous species with a high starch reserve, and because it has no protective lemma and palea.

Three legumes were chosen to represent the dicotyledonous group of plants. Substantial data have established the relative susceptibility of these plants to a number of established herbicides. The data in Table 2 indicate that any one of these legumes would be satisfactory for this test. Alfalfa was chosen because a good supply of seed with a known high germination was at hand.

Data of Table 2 indicate that _Setaria viridis_ and _Avena fatua_ were the only two of the weed species that gave a reasonably high percentage of germination, 58% and 92% respectively. _S. viridis_ was selected because of its small size, and because of its uniform inception of growth and its rapid elongation of the coleoptile.

It is reasonable to assume that the length of time the seed is in contact with the herbicide is important to the results of this test. By referring again to Table 2, it will be noted that at the end of 144 hours the primary root of _S. viridis_ had begun to decay. This would indicate that 144 hours was beyond the optimum period of time for growth under these conditions. At the end of 120 hours of incubation the roots of wheat had become entangled forming a dense mat. This would make it difficult to separate the seedlings so that individual root length determinations could be made. All species had made satisfactory growth at the end of 96 hours of incubation; therefore this period was used uniformly throughout these investigations.

Table 3 presents the range of herbicidal materials used in this test. The active ingredients of the compounds one to four have been widely tested and their value as herbicides is established. The remaining compounds have been tested to a lesser degree so that their relative qualities as herbicides are not well established.

To facilitate mixing some of the more insoluble compounds with water it was necessary to use a wetting agent. Nonic 218 (Sharples Chemicals, Incorporated) was selected for this use. A test was run to determine any indication of growth response due to the action of the wetting agent. Using the technique developed earlier each of the three species of seed was germinated in a solution of 50 ppm of Nonic 218. Checks of distilled water were run simultaneously. Results of this test indicated that the wetting agent did not bring about significant differences in growth over a 96 hour period except in the case of the wheat shoots. Here there was a decrease in shoot length which was significant at the five percent level. Mean shoot length of the treated wheat seedlings of the check was 6.8 mms. whereas the mean length of untreated shoots was 8.2 mms. Because this difference was determined, 50 ppm of the wetting agent were added to all solutions of herbicides to minimize the induced error.

A stock solution of all herbicides was made up at a concentration of 1000 ppm. Concentrations of 10, 100, 500, and 1000 ppm were then developed by proper dilution of the stock solutions. In addition, a check treatment of distilled water was included. Each concentration and the check were represented by four dishes for each species.

Mean length of roots and shoots was determined by taking the average of all seedlings within the individual dishes. Data collected were treated statistically by analysis of variance (11) and the variance

Table 3. List of herbicidal materials, active ingredients, and formulators of the chemicals used in these tests.

	Herbicidal Materials	Active ingredients	Formulator[1]
1	2,4-D	2,4-dichlorophenoxyacetic acid	American Chemical Paint Co.
2	IPC	isopropyl N-phenyl carbamate	J. T. Baker Chemical Co.
3	TCA	sodium trichloroacetate	Dow Chemical Co.
4	XP40A	xanthogen disulfide	Sherwin-Williams Co.
5	EC 3740	disodium 3,6-endoxohexahydrophthalate	Sharples Chemicals, Inc.
6	N-4053	maleic hydrazide	United States Rubber Co.
7	EH 1	Sodium 2,4-dichlorophenyl "Cellosolve" sulfate	Carbide and Carbon Chemicals Corp.
8	EH 1	dichloral urea	Carbide and Carbon Chemicals Corp.
9	EC 3890	sodium trichloroacrylate	Sharples Chemicals, Inc.
10	EH 5667	1,2,2-trichloroacrylamide	Carbide and Carbon Chemicals Corp.
11	EH 568	phenyl "Cellosolve" acetate	Carbide and Carbon Chemicals Corp.
12	EH 5722	phenyl "Cellosolve" trichloroacetate	Carbide and Carbon Chemicals Corp.
13	EH 5665	N(alpha-hydroxy beta-trichloroethyl) benzamide	Carbide and Carbon Chemicals Corp.
14	EH 5678	N(alpha-hydroxy beta-trichloroethyl) chloroacetamide	Carbide and Carbon Chemicals Corp.
15	EH 5731	N(alpha-hydroxy beta-trichloroethyl) trichloroacetamide	Carbide and Carbon Chemicals Corp.
16	EH 5669	reaction product of ethylene urea and chloral hydrate	Carbide and Carbon Chemicals Corp.
17	EH 5476	calcium 2,4-dichlorophenyl "Cellosolve" sulfate	Carbide and Carbon Chemicals Corp.
18	EH Mix	formulated EH 2 and EH 5476	Carbide and Carbon Chemicals Corp.

1/ The author wishes to express his appreciation to the formulators of the materials indicated above for their assistance in this study.

for treatment levels was further subdivided into variance associated with regression of growth on the logarithm of the chemical concentration, and deviation from this regression.

In this regression analysis the data from the check or zero concentration of the chemical could not be used since the presence of the independent variable is essential to any regression study. In plotting the X-scale, concentrations of the chemical in parts per million were expressed as logarithms to the base ten. The Y-scale was expressed in actual measurement units (Griffing). Variance for deviation from regression was obtained by subtraction from the total variance. It follows that the latter category will contain deviations from linearity on the logarithmic scale, as well as error.

Data from a greenhouse screening test being run concurrently were available. These data were compared to those from the laboratory screening test to determine whether or not there is a correlation between the relative toxicities of the various chemicals as evaluated by the two tests.

The plants used in this test included five crop plants, soybeans, field peas, oats, sugar beets, and flax. In addition proso millet (Panicum miliaceum) was grown with the sugar beets and mustard (Brassica arvensis) with the flax. All of the chemicals tested in the laboratory could not be included in this test since the limited space necessitated the omission of a few.

In the greenhouse the chemicals were tested at two rates and were applied as both preemergence and postemergence treatments. The preemergence treatments were conducted on plants planted in rows on a bench; each treatment being separated by fibreboard strips set in the soil. Preemergence treatments were made one day before emergence of the

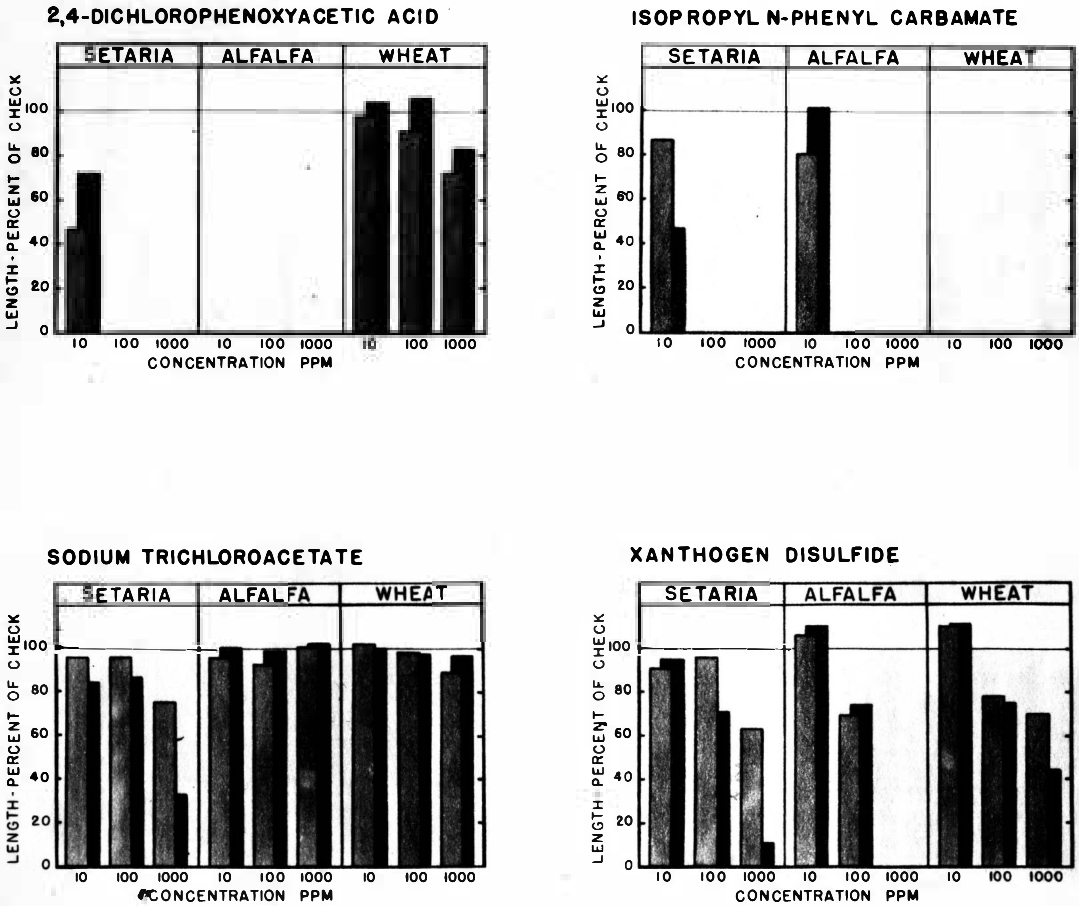

Figure 1. Bar graphs of root and shoot lengths of three species of seeds expressed as a per cent of normal growth as modified by three concentrations of four herbicidal chemicals. Concentrations in ppm. Hatched bar, root growth; solid bar, shoot growth.

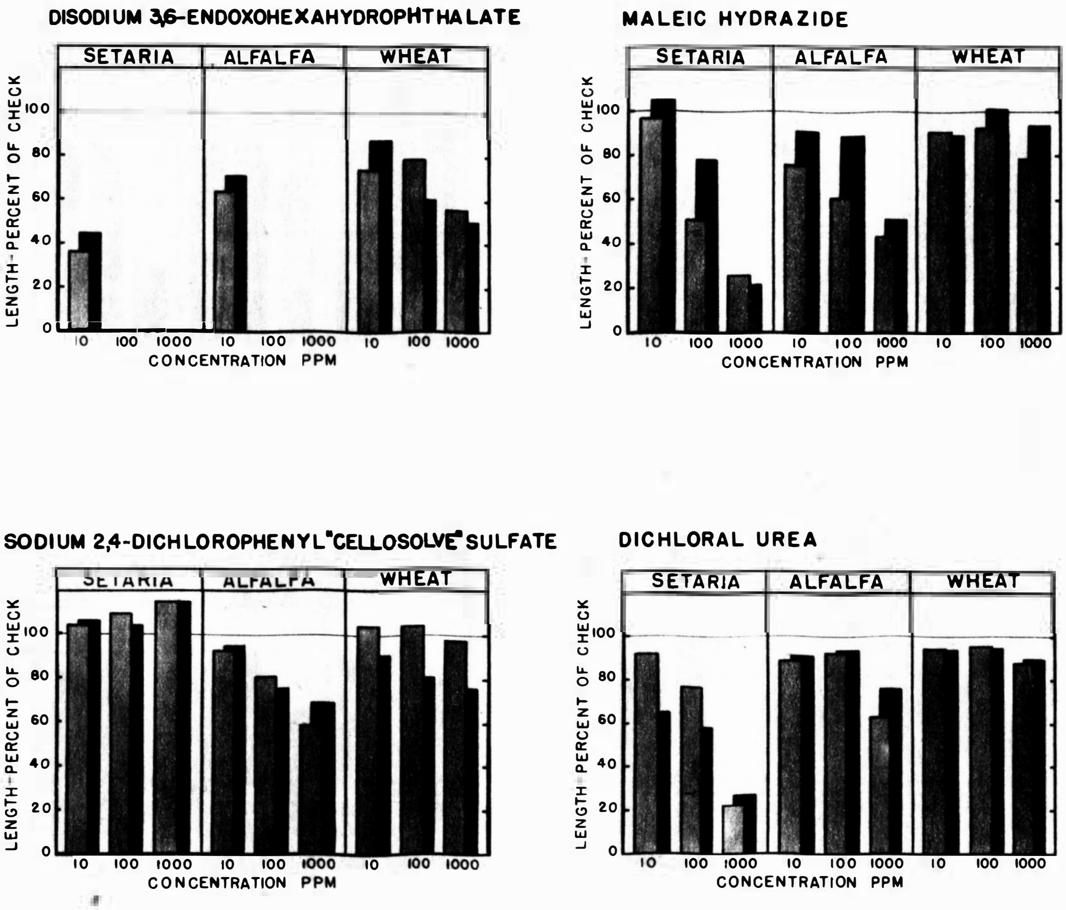

Figure 2. Bar graphs of root and shoot lengths of three species of seeds expressed as a per cent of normal growth as modified by three concentrations of four herbicidal chemicals. Concentrations in ppm. Hatched bar, root growth; solid bar, shoot growth.

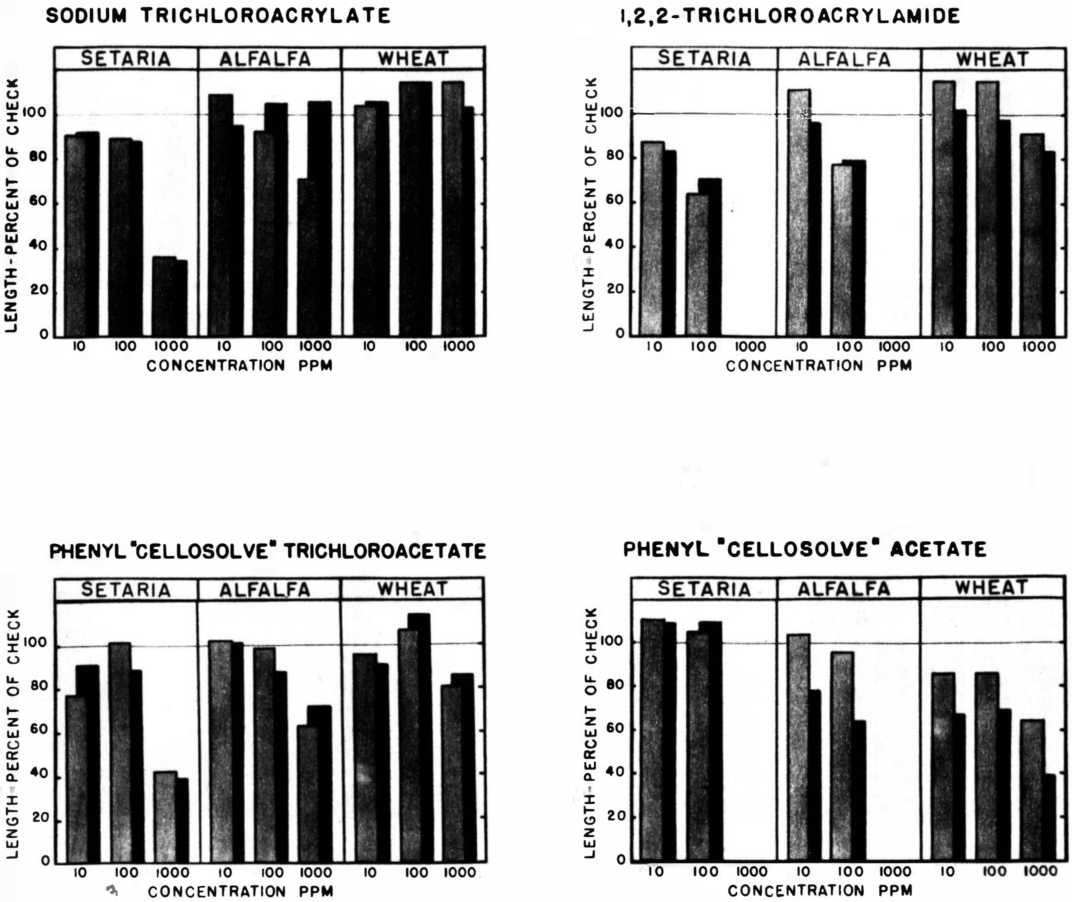

Figure 3. Bar graphs of root and shoot lengths of three species of seeds expressed as a per cent of normal growth as modified by three concentrations of four herbicidal chemicals. Concentrations in ppm. Hatched bar, root growth; solid bar, shoot growth.

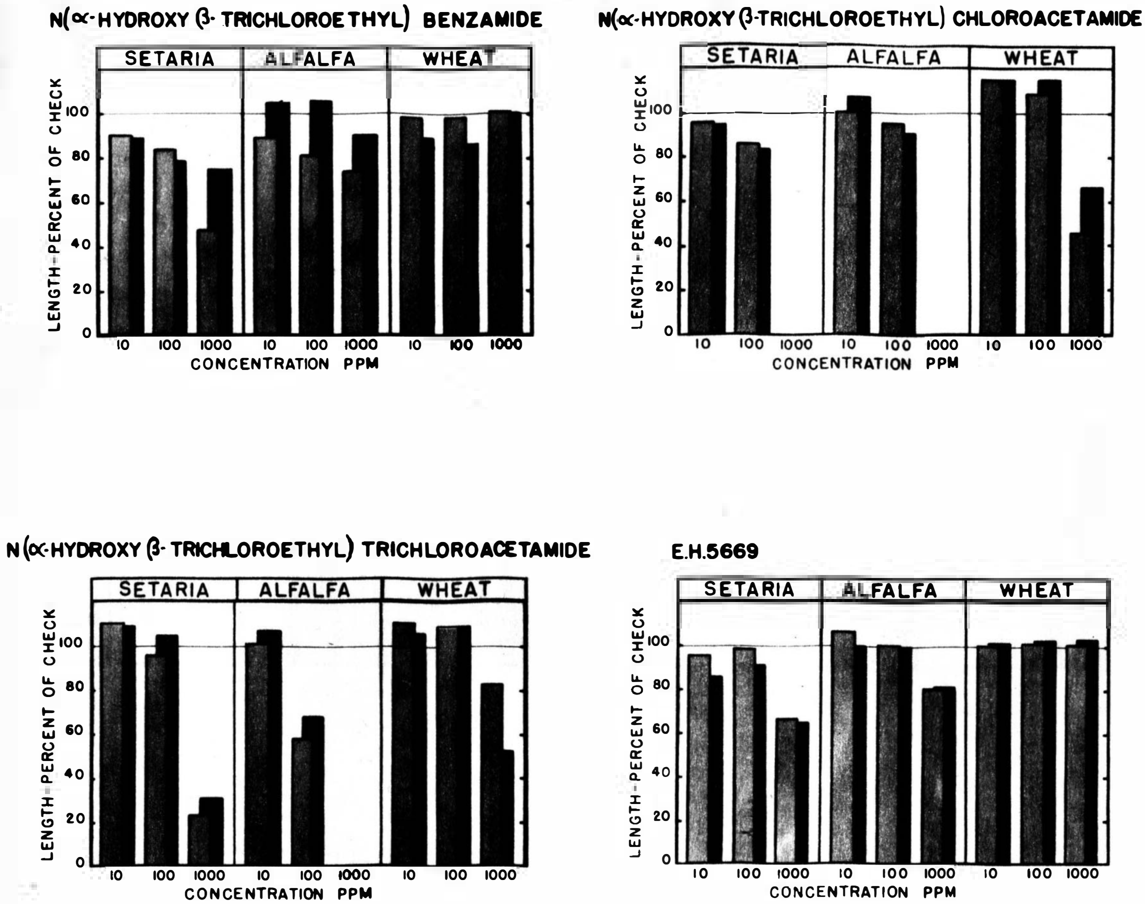

Figure 4. Bar graphs of root and shoot lengths of three species of seeds expressed as a per cent of normal growth as modified by three concentrations of four herbicidal chemicals. Concentrations in ppm. Hatched bar, root growth; solid bar, shoot growth. (N(alpha-hydroxy beta-trichloroethyl) chloroacetamide, shoot growth 10 ppm, 125.3%; 100 ppm 153.3%.)

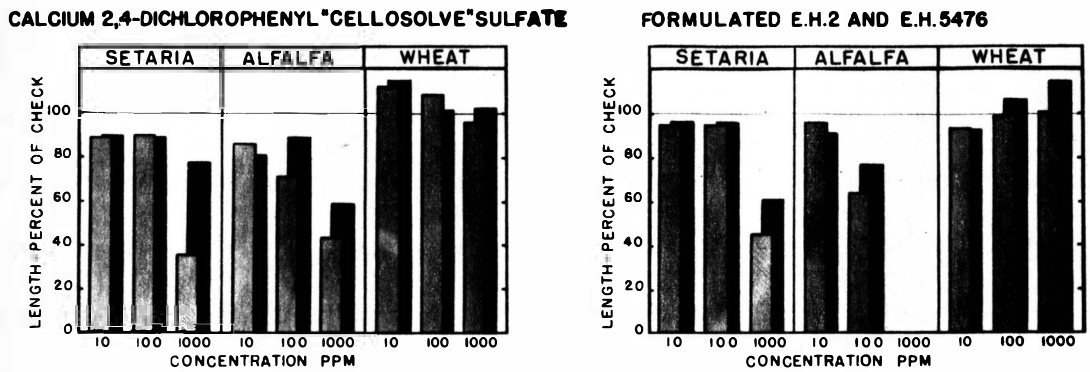

Figure 5. Bar graphs of root and shoot lengths of three species of seeds expressed as a per cent of normal growth as modified by three concentrations of two herbicidal chemicals. Concentrations in ppm. Hatched bar, root growth; solid bar, shoot growth.

plants. Postemergence treatments were applied when the millet seedlings were approximately one inch to one and one-half inches tall. Four weeks after treatments were applied the plants were rated by three people who compared the treated material to the checks and assigned percentages to the former with respect to both stand and vigor.

DATA AND DISCUSSION

Figures 1 to 5 graphically present the reactions of the three species to the test chemicals as recorded in Tables 4,5, and 6. The hatched bars represent shoot length and the solid bars the root length. In each case the respective bars represent the average lengths expressed as a percentage of that determined for the untreated check which was run simultaneously. Although each chemical was tested at four concentrations only three were represented in graphic form since the abscissa was in logarithms and only three of the four concentrations fell at equal intervals on this scale. Where no germination occurred or where growth was highly abnormal no bar was constructed since length determinations were not made in such instances.

Average root and shoot lengths expressed as a percentage of their respective checks are found in Tables 4, 5, and 6. These are discussed with reference to the analyses of variance taking each chemical individually. A mean square for regression larger than the mean square for treatments indicated that a larger portion of the total variance was associated with regression. If for a certain chemical, variance attributable to regression was significant and was accompanied by significant differences at the treatment levels, it was assumed that the chemical possessed growth inhibitory properties. Many of the chemicals included in this test gave an indication of inhibition with respect to one or more of the species. In a few cases where significant differences were indicated at the treatment level but where the average length at the highest concentration was above the check, analysis for regression was not made. Needless to say, when complete suppression of development occurred inhibitory properties were exhibited.

Table 4. Length of root and shoot (percent of check) of Setaria seedlings treated with 18 different chemicals at four concentrations.

Concentration		10 ppm		100 ppm		500 ppm		1000 ppm	
Chemical		% Root	% Shoot	% Root	% Shoot	% Root	% Shoot	% Root	% Shoot
1	2,4-D	47.2	73.4	--[1/]	--	--	--	--	--
2	IPC	87.9	47.0	--	--	--	--	--	--
3	TCA	96.8	85.0	97.3	87.0	93.1	65.4	75.7	33.1
4	XP40A	91.1	95.0	97.7	70.9	63.6	10.1	65.9	12.6
5	EC 3740	36.8	44.8	--	--	--	--	--	--
6	N-4053	97.1	105.2	51.9	78.1	26.6	30.1	25.3	21.6
7	EH 1	104.2	107.8	109.3	104.6	93.9	118.0	121.8	115.4
8	EH 2	93.9	65.5	77.2	58.8	30.9	43.3	22.4	27.8
9	EC 3890	91.5	92.1	88.8	87.4	55.8	54.7	36.2	34.3
10	EH 5667	87.7	83.1	60.8	70.9	--	--	--	--
11	EH 568	111.2	109.1	105.2	108.7	60.2	83.1	--	--
12	EH 5722	77.4	91.0	101.2	88.2	72.0	66.4	42.4	39.6
13	EH 5668	90.6	89.5	84.0	78.9	67.4	82.9	47.9	75.3
14	EH 5678	95.6	94.1	85.8	83.3	27.0	38.3	--	--
15	EH 5731	110.7	109.6	96.8	105.5	44.0	80.9	23.0	32.7
16	EH 5669	95.7	86.5	99.2	92.1	93.8	92.9	67.2	65.0
17	EH 5476	88.9	89.6	90.0	89.6	81.3	92.1	36.0	78.1
18	EH MIX	95.7	97.4	95.7	97.1	68.7	87.5	45.6	61.7

1/ No measurable development

Table 5. Length of root and shoot (percent of check) of alfalfa seedlings treated with 18 different chemicals at four concentrations.

Concentration		10 ppm		100 ppm		500 ppm		1000 ppm	
Chemical		% Root	% Shoot	% Root	% Shoot	% Root	% Shoot	% Root	% Shoot
1	2,4-D	--	--	--	--	--	--	--	--
2	IPC	81.2	102.0	--	--	--	--	--	--
3	TCA	96.4	100.9	92.3	99.1	87.4	99.1	100.4	102.8
4	XP4OA	107.7	110.5	69.9	74.0	--	--	--	--
5	EC 3740	63.4	70.5	--	--	--	--	--	--
6	N-4053	76.5	91.4	61.5	89.2	51.3	66.2	43.8	51.1
7	EH 1	83.1	95.3	80.8	75.8	72.9	64.1	58.6	69.5
8	EH 2	89.3	91.1	93.4	93.8	80.2	87.5	63.5	75.9
9	EC 3890	109.4	95.7	93.3	105.4	78.3	107.6	70.6	105.7
10	EH 5667	111.0	95.9	78.1	79.7	--	--	--	--
11	EH 568	104.7	78.5	95.3	64.6	--	--	--	--
12	EH 5722	103.2	102.4	99.5	87.8	88.2	74.8	63.6	72.4
13	EH 5665	68.9	105.5	81.7	106.4	77.3	91.8	74.9	90.0
14	EH 5678	100.8	107.3	95.5	91.9	--	--	--	--
15	EH 5731	101.9	107.8	58.0	68.9	22.1	84.5	--	--
16	EH 5669	107.6	100.0	101.4	100.0	97.1	95.1	80.0	80.6
17	EH 5476	86.2	82.3	71.5	89.4	43.5	74.5	43.5	59.6
18	EH MIX	96.2	92.4	63.7	77.1	--	--	--	--

Table 6. Length of root and shoot (percent of check) of wheat seedlings treated with 18 different chemicals at four concentrations.

Concentration		10 ppm		100 ppm		500 ppm		1000 ppm	
Chemical		% Root	% Shoot	% Root	% Shoot	% Root	% Shoot	% Root	% Shoot
1	2,4-D	98.9	104.3	91.0	106.4	82.8	91.5	72.0	83.0
2	IPC	--	--	--	--	--	--	--	--
3	TCA	100.2	100.0	98.5	98.2	95.2	107.0	88.4	96.5
4	XP4OA	110.9	111.5	78.2	75.0	60.3	40.4	70.0	44.2
5	EC 3740	74.6	87.5	78.5	60.0	65.9	51.3	55.6	50.0
6	N-4053	90.9	89.5	92.5	101.8	80.7	89.5	79.3	94.7
7	EH 1	104.8	90.0	104.3	80.0	93.9	76.7	97.7	76.7
8	EH 2	94.8	94.1	95.2	94.1	91.1	102.9	87.3	89.7
9	EC 3890	104.4	107.1	120.7	117.9	107.8	103.6	116.0	103.6
10	EH 5667	116.2	102.4	115.6	97.6	111.5	100.0	90.3	82.9
11	EH 568	87.4	67.2	87.4	69.0	86.3	63.8	64.3	39.7
12	EH 5722	96.0	91.3	107.9	114.1	101.3	118.5	80.3	85.9
13	EH 5665	98.3	88.2	98.3	85.3	93.1	92.6	100.8	100.0
14	EH 5678	111.7	125.3	108.6	153.3	68.5	100.0	46.9	66.7
15	EH 5731	110.5	105.4	109.4	109.7	101.5	100.0	83.9	57.6
16	EH 5669	100.9	102.3	102.3	104.7	102.5	123.2	101.7	104.7
17	EH 5476	113.8	124.5	108.1	101.9	99.0	107.5	96.8	103.8
18	EH MIX	92.9	92.3	98.0	107.7	98.8	105.8	101.5	128.8

1. 2,4-D

Setaria - Growth was highly abnormal at rates above 10 ppm. While some shoot development occurred at these rates, no roots emerged and therefore zero germination was recorded. At 10 ppm, the mean root and shoot growth was inhibited by 85% and 30% respectively.

Alfalfa - There was no germination of alfalfa at even the lowest concentration (10 ppm). Seeds were swollen and the seed coats broken but there was no normal development of seedlings.

Wheat - This crop germinated at all concentrations. Average root length was reduced to a highly significant degree. Variation is

Table 7. Analyses of variance of root and shoot length of wheat seedlings treated with 2,4-D at four concentrations.

Source of variation	Root length d.f.		Root length Mean Square	Shoot length d.f.		Shoot length Mean Square
Total	15			15		
Treatment	3		66.25**[1]	3		1.13*
Regression		1	180.86**		1	2.24**
Deviations		2	8.95		2	.58
Error	12		7.25	12		.23

1/In this and following tables *significant at 5% level
**significant at 1% level

apparently associated with regression as indicated by the much larger portion of variance attributable to regression. Shoot length shows differences significant at the 5% level. Variance due to regression, however, is significant at the one percent level.

2. IPC

Setaria - When treated with IPC Setaria seedlings germinated only in the 10 ppm concentrations. Root length was approximately 90% of the check whereas shoot length was only 50% of the untreated shoot growth.

Alfalfa - Alfalfa seeds germinated at 10 ppm but showed a 20% inhibition to the mean root length. Shoot length did not appear to be affected. At 100 ppm seedlings were so abnormal that measurements were not taken. Germination was completely inhibited at higher rates.

Wheat - Wheat failed to develop at all concentrations. At 10 ppm and 100 ppm abnormal growth not exceeding 1/8 of an inch in length appeared but no normal roots or shoots were produced.

3. Xanthogen disulfide

Setaria - Roots and shoots showed marked differences in their responses to XP40A. While highly significant decreases were determined in both cases the shoots were more severely inhibited than were the

Table 8. Analyses of variance of root and shoot length of Setaria seedlings treated with Xanthogen disulfide at four concentrations.

Source of variation	Root length d.f.		Root length Mean square	Shoot length d.f.		Shoot length Mean square
Total	15			15		
Treatment	3		84.30**	3		557.62**
Regression		1	160.79**		1	1525.42**
Deviation		2	46.06		2	73.73
Error	12		7.68	12		6.85

roots. Only 12% of normal shoot growth was determined at 1000 ppm whereas 65% root growth was determined at the same concentration.

Alfalfa - At 100 ppm root and shoot length was approximately 70% of the untreated check. Seedlings produced in dishes containing 500 ppm were severely malformed while seeds in higher concentrations failed to germinate.

Wheat - Wheat seedlings were inhibited to a high degree with a high increase in variance attributable to regression in the case of both roots and shoots (Table 9). At the highest concentration root growth was 70% of the check and the shoot length approximately 40%.

Table 9. Analyses of variance of root and shoot length of wheat seedlings treated with XP40A at four concentrations.

Source of variation	Root length d.f.		Root length Mean square	Shoot length d.f.		Shoot length Mean square
Total	15			15		
Treatment	3		321.60**	3		11.80**
Regression		1	815.13**		1	34.02**
Deviation		2	74.84		2	.69
Error	12		2.90	12		.31

4. Sodium TCA

<u>Setaria</u> - Treatments with sodium TCA brought about significant decreases in root and shoot length and a similar trend regarding regression. There was, however, a marked difference in percent of inhibition

Table 10. Analyses of variance of root and shoot length of Setaria seedlings treated with TCA at four concentrations.

Source of variation	Root length d.f.		Root length Mean square	Shoot length d.f.		Shoot length Mean square
Total	15			15		
Treatment	3		27.57*	3		163.89**
Regression		1	65.49*		1	303.39**
Deviation		2	8.60		2	133.70
Error	12		8.21	12		6.59

to roots in contrast to that of the shoots. At the highest rate root growth was 75% of the check whereas the treatment resulted in a mean shoot length of only 30%.

<u>Alfalfa</u> - Statistical analysis reveals no significant differences in either root or shoot lengths due to treatment at any rate. Analysis for regression was not conducted (Table 11).

Table 11. Analyses of variance of root and shoot length of alfalfa seedlings treated with TCA at four concentrations.

Source of variation	Root length d.f.	Root length Mean square	Shoot length d.f.	Shoot length Mean square
Total	15		15	
Treatment	3	5.94	3	.19
Error	12	4.15	12	5.09

Wheat - Neither the roots nor shoots exhibited any significant decrease due to treatment with TCA (Table 12).

Table 12. Analyses of variance of root and shoot length of wheat seedlings treated with TCA at four consentrations.

Source of variation	Root length d.f.	Root length Mean square	Shoot length d.f.	Shoot length Mean square
Total	15		15	
Treatment	3	22.26	3	.26
Error	12	5.36	12	1.85

5. EC 3740

Setaria - Growth was greatly inhibited by treatment with EC 3740; germination at 100 ppm and above was completely inhibited while at 10 ppm growth was only about 40% of the untreated check.

Alfalfa - Growth of alfalfa was completely inhibited at rates of 100 ppm and above. At 10 ppm growth was about 70% of the check.

Wheat - Wheat seedlings showed more resistance than either Setaria or alfalfa to this treatment but growth was inhibited to a highly significant degree. All rates showed considerably less growth than the check. The lowest rate produced about 80%, and the high rate about 50% of the check. Root and shoot responded similarly. (Table 13).

Table 13. Analyses of variance of root and shoot length of wheat seedlings treated with EC 5740 at four concentrations.

Source of variation	Root length d.f.		Root length Mean square	Shoot length d.f.		Shoot length Mean square
Total	15			15		
Treatment	3		70.24**	3		7.74**
Regression		1	125.65**		1	21.97**
Deviation		2	42.84		2	.64
Error	12		4.45	12		.22

6. N-4053

<u>Setaria</u> - A very large portion of variation was associated with regression (Table 14). Mean square for treatment and for regression were highly significant as a result of N-4053 treatments. While 10 ppm caused

Table 14. Analyses of variance of root and shoot length of Setaria seedlings when treated with maleic hydrazide at four concentrations.

Source of variation	Root length d.f.		Root length Mean square	Shoot length d.f.		Shoot length Mean square
Total	15			15		
Treatment	3		26.07**	3		449.80**
Regression		1	726.36**		1	1282.71**
Deviation		2	27.87		2	38.35
Error	12		1.38	12		22.90

changes not greatly different from the check (Table 4), 1000 ppm resulted in growth approximately 25% of the check; roots and shoots were affected to the same degree.

<u>Alfalfa</u> - Variance associated with treatment and with regression was highly significant for both roots and shoots. All rates of N-4053 inhibited growth of alfalfa seedlings (Table 5). Mean root length at the lowest rate was 76.5% of the check, and 45% at the high rate. Mean shoot length was reduced from 90% at the high rate and 50% at the low rate.

Table 15. Analyses of variance of root and shoot length of alfalfa seedlings when treated with M-4053 at four concentrations.

Source of variation	Root length d.f.	Root length Mean square	Shoot length d.f.	Shoot length Mean square
Total	15		15	
Treatment	3	40.30**	3	28.87**
Regression	1	118.17**	1	69.21**
Deviation	2	1.36	2	8.70
Error	12	1.65	12	3.16

Wheat - There were no significant differences (Table 16) in root or shoot growth between treatments when wheat was grown in solutions of M-4053, at concentrations of from 10 to 1000 ppm.

Table 16. Analyses of variance of root and shoot growth when wheat seedlings were treated with maleic hydrazide at four concentrations.

Source of variation	Root length d.f.	Root length Mean square	Shoot length d.f.	Shoot length Mean square
Total	15		15	
Treatment	3	43.17	3	.45
Error	12	13.01	12	.23

7. EH 1

Setaria - Mean root length of Setaria exhibited no significant differences when grown in solutions of EH 1 (Table 17). Average shoot length of Setaria was significantly different at the 5% level. Analysis

Table 17. Analyses of variance of mean root and shoot length of Setaria seedlings when treated with EH 1 at four concentrations.

Source of variation	Root length d.f.	Root length Mean square	Shoot length d.f.	Shoot length Mean square
Total	15		15	
Treatment	3	37.81	3	15.01
Error	12	12.88	12	3.31

was not made for regression because most measurements were above the check. At 500 ppm shoot growth was greatest with 118% of the check and

115.4% at 1000 ppm (Table 4).

Alfalfa - Analysis of variance of mean root length of alfalfa (Table 18) showed high significant differences between treatments with EH 1. Variance attributable to regression was great. Reduction of shoot growth was not great enough to bring about significant differences. Mean square for regression was, however, large enough to be statistically significant at the 5% level.

Table 18. Analysis of variance of root and shoot length of alfalfa when treated with EH 1 at four concentrations.

Source of variation	Root length d.f.	Root length Mean square	Shoot length d.f.	Shoot length Mean square
Total	15		15	
Treatment	3	34.59**	3	12.12
Regression	1	95.08**	1	24.73*
Deviation	2	15.47	2	5.81
Error	12	5.27	12	4.89

Wheat - Differences significant at the 5% level (Table 19) were apparent in analysis of root growth as a result of treatment of wheat with EH 1. Variance associated with regression was also significant even though inhibition at the highest concentration was 2.3% for roots (Table 6). Mean shoot length showed no significant difference as a result of treatment of wheat seedlings with EH 1.

Table 19. Analyses of variance of root and shoot length of wheat seedlings when treated with EH 1 at four concentrations.

Source of variation	Root length d.f.	Root length Mean square	Shoot length d.f.	Shoot length Mean square
Total	15		15	
Treatment	3	17.22*	3	.58
Regression	1	34.47*		
Deviation	2	8.60		
Error	12	4.87	12	.54

8. EH 2

Setaria - Average lengths of both root and shoot show high significant differences between rates due to treatment with EH 2 (Table 19). Variance due to regression was great in both cases. Root growth was

Table 19-A. Analyses of variance of root and shoot length of Setaria seedlings when treated with EH 2 at four concentrations.

Source of variation	Root length		Shoot length	
	d.f.	Mean square	d.f.	Mean square
Total	15		15	
Treatment	3	303.44**	3	91.63**
Regression	1	811.41**	1	223.37**
Deviation	2	45.00	2	51.52
Error	12	4.16	12	8.52

over 90% at the low rate and about 20% at the high rate while for similar rates shoot growth was reduced 65% and 25% respectively (Table 4).

Alfalfa - There were no significant differences between concentrations when alfalfa was treated with EH 2 (Table 20).

Table 20. Analyses of variance of root and shoot length of alfalfa seedlings treated with EH 2 at four concentrations.

Source of variation	Root length		Shoot length	
	d.f.	Mean square	d.f.	Mean square
Total	15			
Treatment	3	34.02		3.08
Error	12	9.98		2.34

Wheat - Mean square associated with root length or shoot length was not great enough to indicate statistical significance (Table 21).

Table 21. Analyses of variance of root and shoot length of wheat seedlings treated with EH 2 at four concentrations.

Source of variation	Root length		Shoot length	
	d.f.	Mean square	d.f.	Mean square
Total	15			
Treatment	3	13.06		1.58
Error	12	16.12		1.33

9. EC 3890

Setaria - Analyses of variance of both root and shoot (Table 22) indicate that a large portion of variance is attributable to regression. At the low rate growth was slightly over 90% (Table 4) whereas

Table 22. Analyses of variance of root and shoot length of Setaria seedlings when treated with EC 3890 at four concentrations.

Source of variation	Root length d.f.		Root length Mean square	Shoot length d.f.		Shoot length Mean square
Total	15			15		
Treatment	3		205.60**	3		194.91**
Regression		1	467.61**		1	482.37**
Deviation		2	74.67		2	51.18
Error	12		5.59	12		8.12

growth at 1000 ppm was only 36% of the untreated checks. The differences were highly significant.

Alfalfa - Mean shoot growth was not affected to a significant degree (Table 23) by this treatment, however, root length showed significant

Table 23. Analyses of variance of root and shoot length of alfalfa seedlings when treated with EC 3890 at four concentrations.

Source of variation	Root length d.f.		Root length Mean square	Shoot length d.f.	Shoot length Mean square
Total	15			15	
Treatment	3		38.18*	3	.98
Regression		1	107.71**		
Deviation		2	3.18		
Error	12		6.47	12	2.48

differences at the 5% level. Variance associated with regression was highly significant for the root measurements.

Wheat - There were no significant differences in root or shoot length due to treatment of wheat with EC 3890 (Table 24).

Table 24. Analyses of variance of root and shoot length of wheat seedlings when treated with EC 3890.

Source of variation	Root length d.f.	Root length Mean square	Shoot length d.f.	Shoot length Mean square
Total	15		15	
Treatment	3	27.76	3	.54
Error	12	16.83	12	.53

10. EH 5667

Setaria - Seedlings failed to develop at rates of 500 ppm and above (Table 4). Root and shoot length of seedlings treated with 10 ppm were over 80% of that produced by the check, but at 100 ppm growth was about 60% of the untreated check.

Alfalfa - At 500 ppm and at 1000 ppm alfalfa seedlings did not develop. Root growth was above that of the check at 10 ppm (111.0%) and about 80% at 100 ppm. Shoot length was 95.9% and 79.7% for 10 ppm and 100 ppm respectively (Table 5).

Wheat - While analysis of variance (Table 6) indicates a high significant difference between treatments there was not a great deal of inhibition (10%) even at the high rate. ~~(10%)~~. At 10, 100, 500 ppm growth was somewhat greater than the check (116.2%, 115.6% and 111.5% respectively). At each concentration the percent of growth of shoots

Table 25. Analyses of variance of root and shoot length of wheat seedlings when treated with EH 5667 at four concentrations.

Source of variation	Root length d.f.	Root length Mean square	Shoot length d.f.	Shoot length Mean square
Total	15		15	
Treatment	3	77.60**	3	.46**
Regression	1	98.17**	1	.78**
Deviation	2	69.79	2	.30
Error	12	6.96	12	.03

was less than that of the roots. There was no stimulation of shoot growth.

11. EH 568

Setaria - Germination at 1000 ppm was zero for this treatment so that it was unnecessary to run an analysis of variance. Lengths at 10 and 100 ppm slightly exceeded the check but at 500 ppm growth was inhibited to such a degree that mean root length was only 60% (Table 4) of the mean root length for the check, and the mean shoot length was somewhat above 80%.

Alfalfa - Growth was not initiated at concentrations of 500 ppm and 1000 ppm when alfalfa was treated with EH 568 (Table 5). At 100 ppm mean shoot length of alfalfa was 95.3% of the check but shoot length was 64.6% of the check.

Wheat - Mean lengths of root and shoot were below the corresponding measurements of the check at all concentrations. Variance associated with regression was significant; for roots at the 5% level and for

Table 26. Analyses of variance of root and shoot length of wheat seedlings when treated with EH 568 at four concentrations.

Source of variation	Root length d.f.	Root length Mean square	Shoot length d.f.	Shoot length Mean square
Total	15		15	
Treatment	3	59.94*	3	2.46**
Regression	1	77.00*	1	3.51**
Deviation	2	51.42	2	1.93
Error	12	11.69	12	.17

shoots at the 1% level (Table 26). As the concentration was increased from 10 ppm to 1000 ppm mean root length dropped from 87.4% to 63.3% of the check and shoot length dropped from 67.2% to 39.7% (Table 6).

12. EH 5722

Setaria - Analysis of variance reveals high significant differences in both root and shoot length (Table 27). At the high rate roots and shoots made about 40% of the growth made by the checks (Table 4). It will be noted that for roots the portion of variance associated with regression was not greater than that for treatments. Variance attributable to regression for shoots was greater than that for treatments.

Table 27. Analyses of variance for roots and shoots of Setaria seedlings when treated with EH 5722 at four concentrations.

Source of variation	Root length		Shoot length	
	d.f.	Mean square	d.f.	Mean square
Total	15		15	
Treatment	3	154.98**	3	195.80**
Regression	1	144.08**	1	366.25**
Deviation	2	320.86	2	110.58
Error	12	3.39	12	7.41

Alfalfa - Growth of alfalfa was somewhat inhibited as a result of treatment with EH 5722. Growth of roots and shoots both showed significance for regression and highly significant differences (Table 28). Seventy percent of the check was the amount of growth made by alfalfa at the highest rate (Table 5).

Table 28. Analyses of variance of roots and shoots of alfalfa seedlings when treated with EH 5722 at four concentrations.

Source of variation	Root length		Shoot length	
	d.f.	Mean square	d.f.	Mean square
Total	15		15	
Treatment	3	43.76**	3	25.39**
Regression	1	93.26**	1	34.47**
Deviation	2	19.01	2	20.56
Error	12	.89	12	1.85

Wheat - Wheat exhibited a variable response to treatment with EH 5722. There were highly significant differences in root growth but variance due to regression was considerably less than variance to treatment (Table 29). Shoot growth was likewise variable but showed no significant differences.

Table 29. Analyses of variance of roots and shoots of wheat seedlings when treated with EH 5722 at four concentrations.

Source of variation	Root length d.f.		Root length Mean square	Shoot length d.f.	Shoot length Mean square
Total	15			15	
Treatment	3		125.82**	3	8.85
Regression		1	57.02		
Deviation		2	160.23		
Error	12		14.01	12	4.37

13. EH 5665

Setaria - The growth of roots produced after treatment with EH 5665 possessed highly significant differences, but the growth of the shoots showed no significant differences (Table 30). Variance associated with regression of shoot length was not significant. Root growth at the 1000 ppm was slightly less than 50% of the check (Table 4).

Table 30. Analyses of variance of root and shoot length of Setaria seedlings when treated with EH 5665 at four concentrations.

Source of variation	Root length d.f.		Root length Mean square	Shoot length d.f.		Shoot length Mean square
Total	15			15		
Treatment	3		120.32**	3		12.88
Regression		1	300.26**		1	26.18
Deviation		2	30.35		2	6.40
Error	12		3.25	12		9.97

Alfalfa - Alfalfa roots were inhibited to a highly significant degree as a result of treatment with EH 5665 (Table 31). Variance attributable to regression was also highly significant. Shoots of alfalfa did not show differences that were statistically significant. In no case was inhibition very great for at the highest rate alfalfa roots were 75% of the check and shoots length were 90% of the check (Table 5).

Table 31. Analyses of variance of root and shoot lengths of alfalfa seedlings when treated with EH 5665 at four concentrations.

Source of variation	Root length d.f.		Root length Mean square	Shoot length d.f.		Shoot length Mean square
Total	15			15		
Treatment	3		10.88**	3		3.48
Regression		1	28.21**		1	8.20
Deviation		2	2.22		2	1.12
Error	12		1.31	12		1.90

__Wheat__ - The shoots of wheat showed differences significant at the 5% level, however, the growth produced by the highest rate was equal to that of the check (Table 6). Unlike the shoots, roots of wheat showed no significant differences (Table 32).

Table 32. Analyses of variance of root and shoot length of wheat seedlings when treated with EH 5665 at four concentrations.

Source of variation	Root length d.f.	Root length Mean square	Shoot length d.f.	Shoot length Mean square
Total	15		15	
Treatment	3	8.08	3	15.06*
Error	12	6.39	12	3.21

14. EH 5678

__Setaria__ - Since there was no germination at 1000 ppm as a result of this treatment the data were not analyzed statistically. Mean lengths were below that of the check in all cases. Root length and shoot length was approximately 5% below the check at the high rate and 27% and 38% for root length and shoot length respectively at the high rate (Table 4).

__Alfalfa__ - No seed germinated normally at 500 ppm nor at 1000 ppm. Growth at the two lower rates was not greatly inhibited. Root and shoot length at 10 and 100 ppm exceeded 90% of the respective lengths produced by the check (Table 5).

__Wheat__ - The growth of wheat was highly irregular at different rates of this treatment. While root growth was stimulated at the two lower rates there was a relatively severe inhibition at the higher rates. Mean root length was less than 50% of the check at the high rate (Table 6). Shoot growth was stimulated considerably at the two lower rates especially at 100 ppm where mean shoot growth was 153.3% of that produced by the check. The amount of shoot growth produced at all three of the other concentrations was lower than 100 ppm. At the high rate only 66.7% of

Table 33. Analyses of variance of root and shoot length of seedlings of wheat when treated with EH 5678 at four concentrations.

Source of variation	Root length d.f.		Root length Mean square	Shoot length d.f.		Shoot length Mean square
Total	15			15		
Treatment	3		963.53**	3		31.48**
Regression		1	2329.13**		1	42.34**
Deviation		2	280.73		2	26.05
Error	12		6.11	12		1.48

the check was produced. A much greater portion of the mean square (Table 33) was associated with regression in the analysis of root length than in the analysis of shoot length. This was partly due to the fact that shoot growth was stimulated up to 100 ppm after which inhibition occurred.

15. EH 5731

Setaria - Variance due to treatment and variance due to regression

Table 34. Analyses of variance of root and shoot length of Setaria seedlings when treated with EH 5731 at four concentrations.

Source of variation	Root length d.f.		Root length Mean square	Shoot length d.f.		Shoot length Mean square
Total	15			15		
Treatment	3		441.78**	3		368.67**
Regression		1	1179.49**		1	757.46**
Deviation		2	49.35		2	174.27
Error	12		6.77	12		7.42

were both highly significant when Setaria was treated with EH 5731 (Table 34).

Root length at the highest rate was 20% of the check while shoot length was about 30% (Table 4).

Alfalfa - Data were not analyzed statistically when alfalfa was treated with EH 5731. Growth initiated at the high rate was very abnormal, therefore lengths were not taken. At 500 ppm root growth was approximately 20% of the check while shoot growth was over 80% (Table 5).

Wheat - Inhibition of wheat seedlings was apparent at 1000 ppm only. At this rate the roots were 83.9% of the check and the shoots which were apparently more severely inhibited made growth amounting to only 57.6% of the check.

Table 34-A. Analyses of variance of root and shoot length of wheat seedlings when treated with EH 5731 at four concentrations.

Source of variation	Root length d.f.		Root length Mean square	Shoot length d.f.		Shoot length Mean square
Total	15			15		
Treatment	3		458.00**	3		19.71**
Regression		1	270.80**		1	26.71**
Deviation		2	551.60		2	12.21
Error	12		5.40	12		1.56

16. EH 5669

Setaria - Highly significant differences for treatments were revealed by statistical analysis of both roots and shoots of Setaria (Table 35). While growth (root and shoot) was above 90% at 500 ppm growth at 1000 ppm was only 65% of the check (Table 4).

Table 35. Analyses of variance of root and shoot length of Setaria seedlings when treated with EH 5669 at four concentrations.

Source of variation	Root length d.f.		Root length Mean square	Shoot length d.f.		Shoot length Mean square
Total	15			15		
Treatment	3		57.08**	3		47.85**
Regression		1	70.05**		1	28.49*
Deviation		2	50.59		2	57.54
Error	12		1.43	12		5.37

Alfalfa - No marked differences were observed as a result of treatment of alfalfa seedlings with EH 5669 except at the high rate; root and shoot growth at the highest rate was 80% of the check (Table 5). Statistical analysis indicate significant differences at the treatment level (Table 36).

Table 36. Analyses of variance of root and shoot length of alfalfa seedlings when treated with EH 5669 at four concentrations.

Source of variation	Root length d.f.	Root length Mean square	Shoot length d.f.	Shoot length Mean square
Total	15		15	
Treatment	3	24.98**	3	3.64*
Regression	1	55.26**	1	6.18*
Deviation	2	9.84	2	2.37
Error	12	3.92	12	.90

Wheat - Mean root and shoot length measurements showed no statistically significant differences as a result of treatment with EH 5669 (Table 37).

Table 37. Analyses of variance of root and shoot length of wheat seedlings when treated with EH 5669 at four concentrations.

Source of variation	Root length d.f.	Root length Mean square	Shoot length d.f.	Shoot length Mean square
Total	15		15	
Treatment	3	.34	3	.77
Error	12	3.87	12	.30

17. EH 5476

Setaria - No significant differences were found when shoot length was analyzed for variance (Table 38). In contrast to this, however, when the root length was analyzed mean square associated with treatment was highly significant; the largest portion of the mean square was associated with regression. Roots at 1000 ppm were less than 40% of the check (Table 4).

Table 38. Analyses of variance of root and shoot length of Setaria when treated with EH 5476 at four concentrations.

Source of variation	Root length d.f.		Root length Mean square	Shoot length d.f.		Shoot length Mean square
Total	15			15		
Treatment	3		219.92**	3		12.02
Regression		1	325.12**		1	8.89
Deviation		2	167.32		2	13.59
Error	12		8.83	12		6.38

Alfalfa - Growth of root and shoots of alfalfa was inhibited so that variance was significant at the one percent level (Table 5). Growth at the low rate was somewhat over 80% while at the high rate root growth was about 40% of the check and shoot length was close to 60%.

Table 39. Analyses of variance of root and shoot length of alfalfa seedlings when treated with EH 5476 at four concentrations.

Source of variation	Root length d.f.		Root length Mean square	Shoot length d.f.		Shoot length Mean square
Total	15			15		
Treatment	3		106.30**	3		13.27**
Regression		1	293.36**		1	19.36**
Deviation		2	12.75		2	10.22
Error	12		4.71	12		.78

Wheat - Differences between average length of both root and shoot show significance at the 5% level (Table 40). However, at the highest rate mean root length of wheat was only 5% below the check and mean shoot

Table 40. Analyses of variance of root and shoot length of wheat seedlings when treated with EH 5476 at four concentrations.

Source of variation	Root length d.f.		Root length Mean square	Shoot length d.f.		Shoot length Mean square
Total	15			15		
Treatment	3		43.17*	3		1.24*
Regression		1	120.16**			
Deviation		2	4.68			
Error	12		8.80	12		.32

length exceeded that of the check (Table 6).

18. EH Mix

Setaria - Setaria roots showed differences significant at the one percent level while differences in shoot length were significant at the 5% level only (Table 41). Both exhibited a large portion of variance due to regression but at the high rate roots were only 45.5% of the check whereas shoots showed about 60% (Table 4).

Table 41. Analyses of variance of root and shoot length of Setaria seedlings when treated with EH Mix at four concentrations.

Source of variation	Root length d.f.		Root length Mean square	Shoot length d.f.		Shoot length Mean square
Total	15			15		
Treatment	3		186.28**	3		109.27**
Regression		1	412.47**		1	199.53**
Deviation		2	73.20		2	64.15
Error	12		15.52	12		15.11

Alfalfa - Alfalfa failed to germinate at 500 ppm and at 1000 ppm; growth (roots and shoots) at 100 ppm was about 70% of the check (Table 5). Growth at 10 ppm was not greatly different from that of the check.

Wheat - Analysis of variance reveals no significant inhibition between treatments to either roots or shoots of wheat when treated with this mixture (Table 42).

Table 42. Analyses of variance of root and shoot length of wheat seedlings treated with EH Mix at four concentrations.

Source of variation	Root length d.f.	Root length Mean square	Shoot length d.f.	Shoot length Mean square
Total	15		15	
Treatment	3	8.20	3	2.30
Error	12	16.90	12	.77

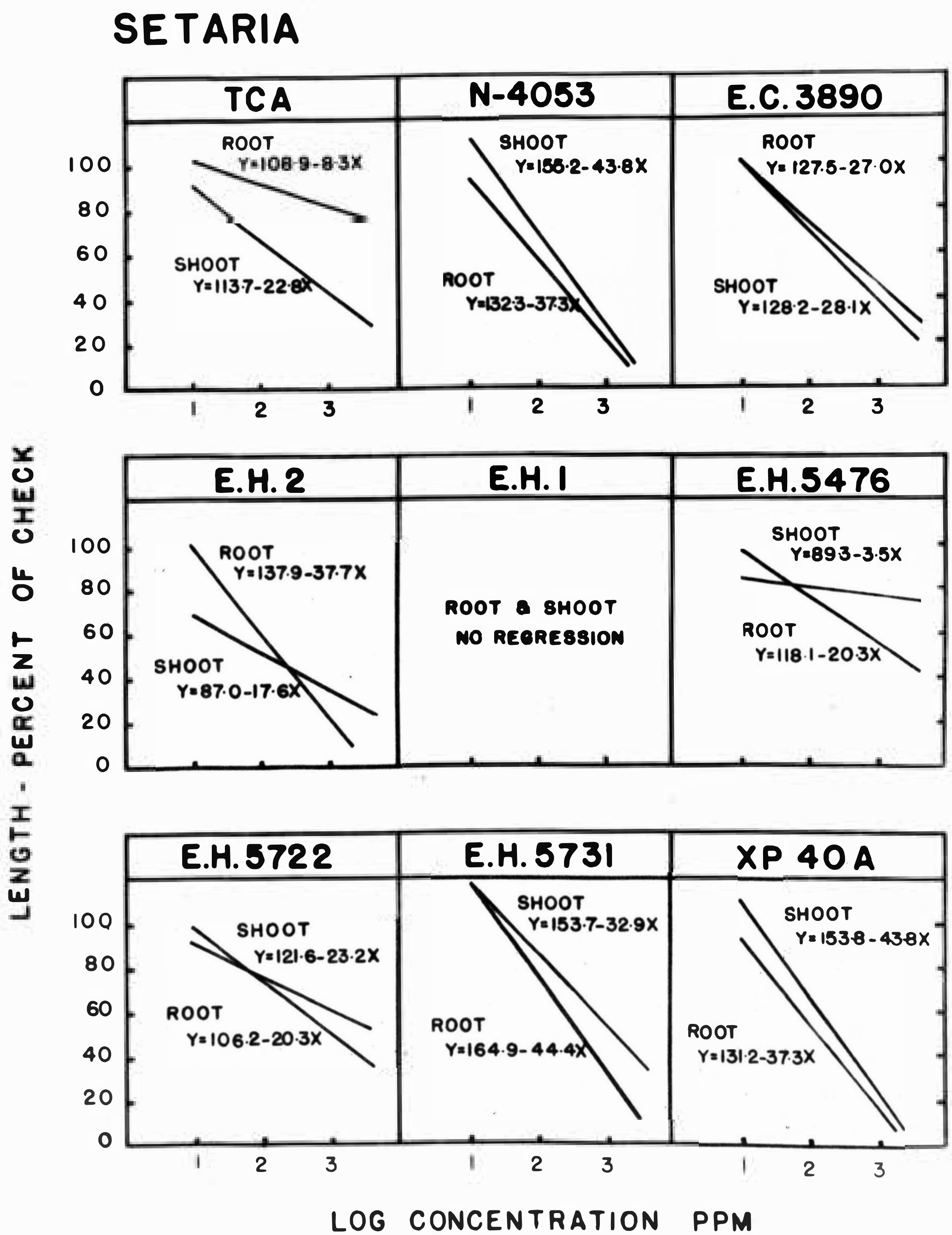

Figure 6. Regression lines for root and shoot length of Setaria seedlings as a per cent of normal growth made by check against concentration (logarithm of ppm) for 9 chemicals.

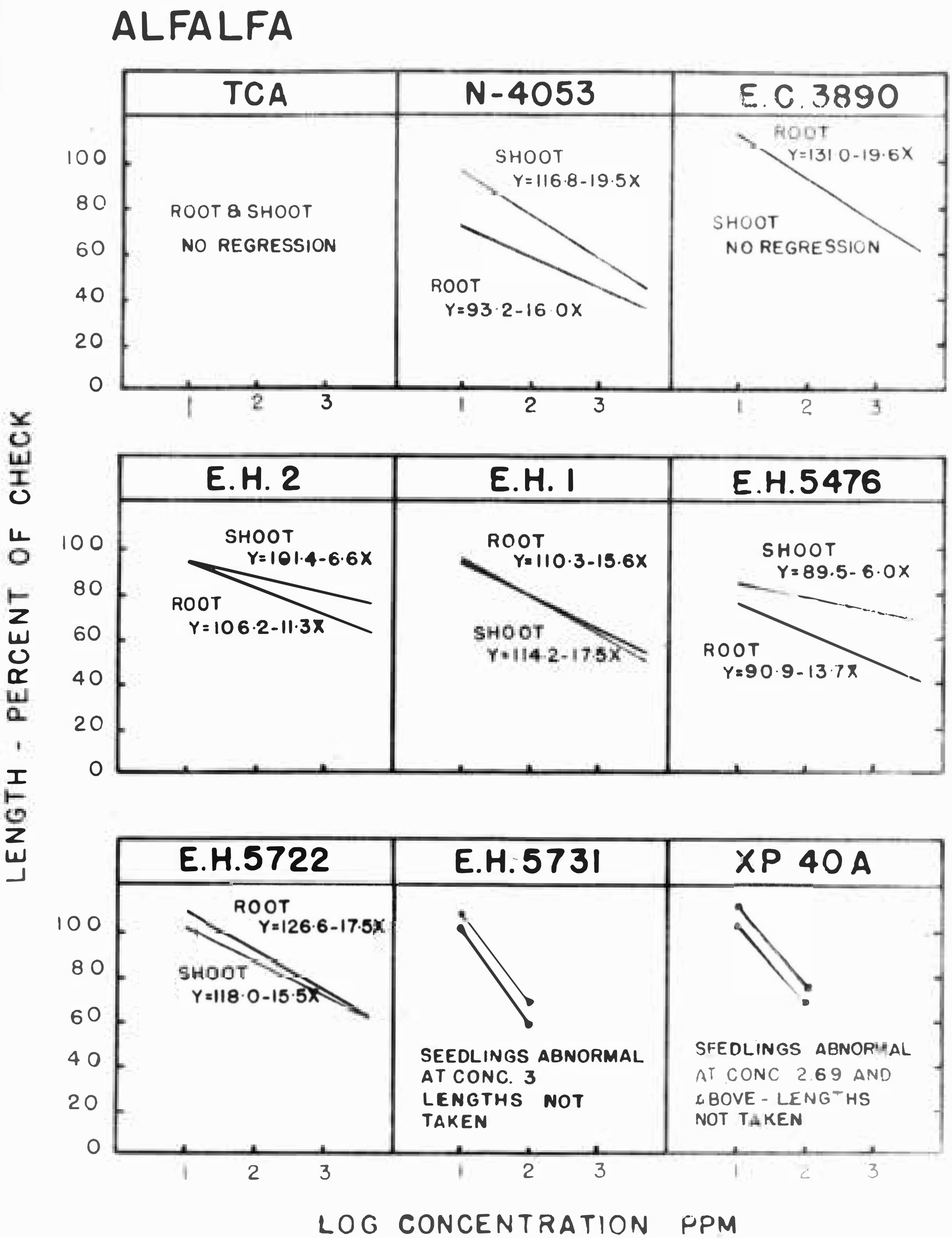

Figure 7. Regression lines for root and shoot length of alfalfa seedlings as a per cent of normal growth made by check against concentration (logarithm of ppm) for six chemicals. Also observations on three chemical compounds for which no regression for seedling growth was established.

It was further desirable to make a relative comparison of those compounds showing strong inhibitory action on one or more of the three plant species tested. For an expression of this evaluation regression coefficients and regression lines were established. Since deviation from regression may in part be attributed to a quadric response; as is suggested by an examination of the bar graphs, (Fig. 1-5), and since this deviation in some cases was of considerable magnitude, it would be unwise to suggest that regression coefficients or regression lines be used for predictions beyond the range of concentration to which the test was restricted. The evaluations made apply only within the range of 10-1000 ppm concentrations of the respective materials.

Figures 6 and 7 present the regression lines with equations (including the coefficients) for selected chemical compounds as they apply to alfalfa and Setaria where analysis of variance indicates inhibition. For the sake of comparison of the reactions of species and the reaction of root and shoot growth of the species, the lengths were expressed as a per cent of the check respectively. The regression coefficient expresses the proportional logarithmic increase in inhibition above the lowest concentration in the test. The inhibition at the lowest concentration is determined by the Y value on the regression line where X equals the log concentration of the lowest concentration. Both these factors, the toxicity at the lower concentration and the increase beyond that point, must be considered when comparing the reaction of species or reactions caused by chemicals.

TCA

Neither the root nor the shoot growth of alfalfa seedlings treated with TCA showed regression but Setaria seedlings treated similarly showed regression for both. At the time of this writing the use of TCA is recommended for the control of certain annual grass seedlings in some broad leaf crops as would seem possible from these data. The regression coefficient of the root growth of Setaria was -8.3 while that of the shoot was -22.8, indicating that the roots were not inhibited to as great an extent as were the shoots within this range.

N-4053

Regression coefficients indicate that within the test range there was a more definite increase in inhibition for Setaria than for alfalfa; however, at the lowest concentration the ordinates of both roots and shoots were less for alfalfa than for Setaria. No marked difference in the sensitivity of the two species was apparent. Regression coefficients of the root did not differ greatly from those of the shoot for either species.

NC 3890

The regression coefficients for the root and for the shoot growth of Setaria were nearly equal. This was in contrast to the wide difference in the corresponding regression coefficients for alfalfa. The shoots of alfalfa showed no regression whereas the roots had a regression coefficient of -10.6.

EH 2

For Setaria, the large regression coefficients of the roots and the position of the regression line for shoots suggested a moderate toxicity of EH 2 for this species. The regression coefficients for alfalfa when treated with EH 2 are comparatively small. A possible selectivity between species was suggested which agreed with work done by King (6).

EH 1

Setaria seedlings treated with EH 1 showed no significance at the treatment levels (Table 17). Regression did exist when data of alfalfa were treated statistically. Regression coefficients of -15.6 and -17.5 for root and shoots respectively were obtained. This differential response is in accord with work done by King (6).

EH 5476

There was considerable difference between the regression coefficients for root and for the shoot growth of Setaria when treated with EH 5476 (Figure 6). Alfalfa shoots were inhibited to a lesser degree than were the roots as is indicated by the regression coefficients.

EH 5722

The regression coefficients for root and shoot growth of Setaria were nearly equal, and both were greater than those for alfalfa. At these concentrations there was only a narrow margin of differential reaction.

EH 5731

Regression coefficients for alfalfa growth could not be determined since measurements above log concentration 2 were not taken because of insufficient development. A line was drawn joining the length percentages where growth was measurable. Quite large regression coefficients were obtained for both root and shoot growth of Setaria seedlings. Both species were affected by this chemical but alfalfa showed the greatest inhibition.

XP40A

For alfalfa growth regression coefficients and regression lines could not be determined since growth above log concentration 2.69 (500 ppm) was not measurable. Lines were drawn joining the length percentages where determinations were possible. Large regression coefficients were obtained for both root and shoot growth of Setaria seedlings treated with XP40A. It was concluded that both species were susceptible to treatment with this chemical with the Setaria seedlings being slightly more tolerant.

Since it was desirable to compare results of the laboratory test with those from a greenhouse trial it was necessary to rate the reactions in

Figure 8. Top row left, peas; right, soybeans. Bottom row left, flax; right, sugar beets and proso millet. Treated post emergence with IPC at low rate 2 pounds per acre, high rate 4 pounds per acre.

Figure 9. Top row left, peas; right, soybeans. Bottom row left, flax and mustard; right sugar beets and proso millet. Treated post emergence with EC 3740 at low rates, 1 pound per acre, high rate 2 pounds per acre.


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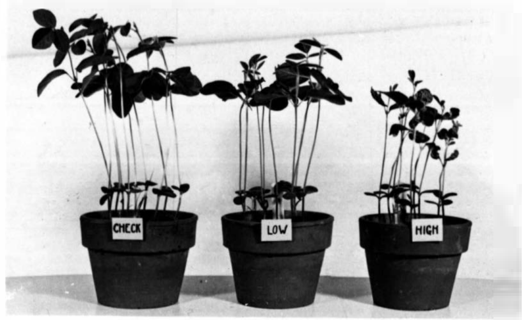
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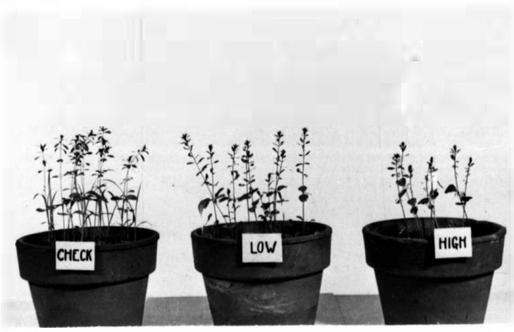
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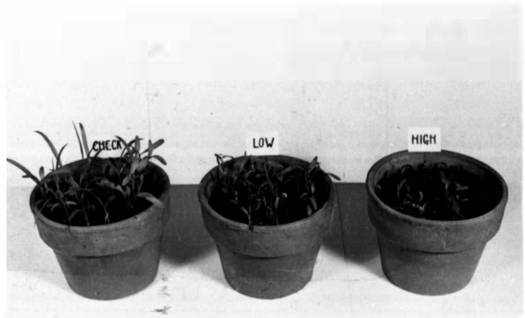
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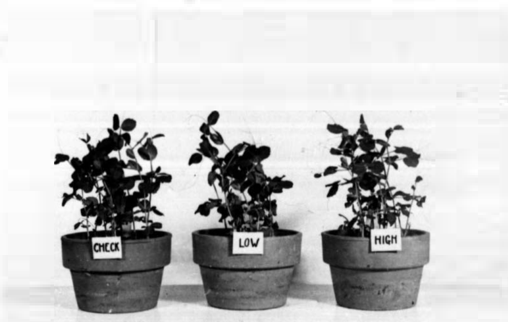
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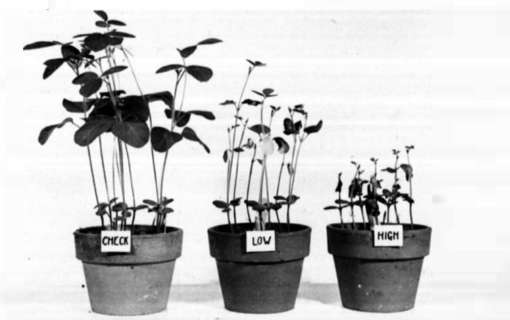
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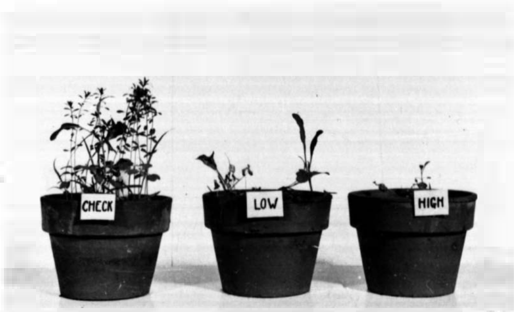
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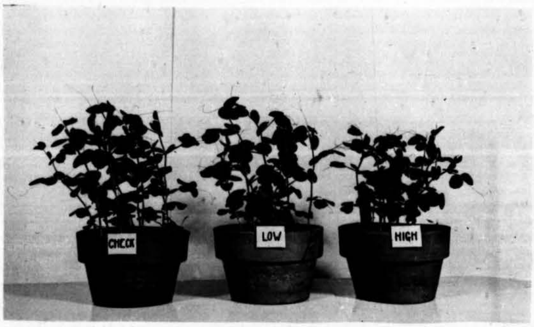
Figure 10. Top row left, peas; right, soybeans. Bottom row left, flax and mustard; right sugar beets and proso millet. Treated post emergence with EC 3390 at low rate 2 pounds per acre, high rate 4 pounds per acre.

Figure 11. Top row left, peas; right, soybeans. Bottom row left, flax and mustard; right sugar beets and proso millet. Treated post emergence with EH Mix at low rate 2 pounds per acre, high rate 4 pounds per acre.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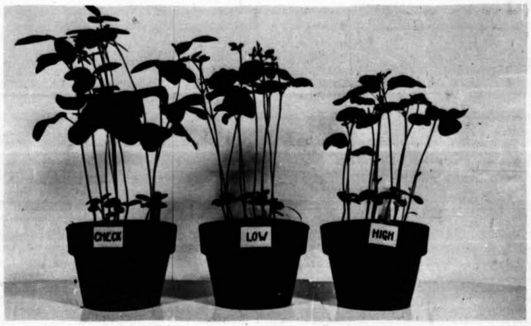
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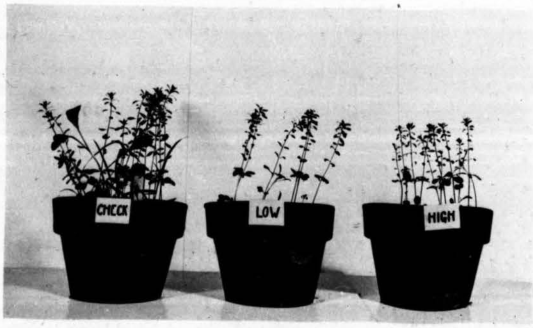
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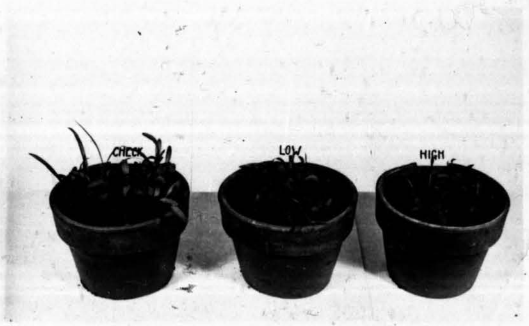
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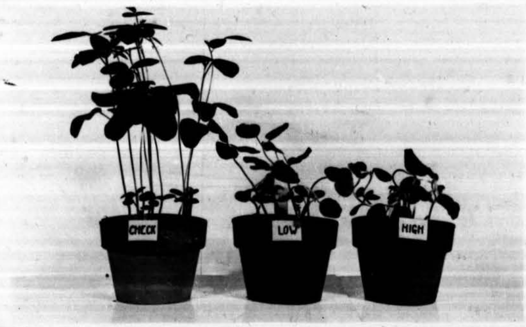
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Table 43. Comparison of results of germination tests and greenhouse tests of herbicidal compounds on the basis of performance ratings assigned for treatments with 18 compounds.

Chemical	Germination test			Greenhouse	
	Setaria	alfalfa	wheat	Score	Rating
1. 2,4-D	xxx	xxxx	x	N.D.	N.D.
2. IPC	xxx	xxx	xxxx	1570	****
3. TCA	xx	--	--	2180	*** 2/
4. XP40A	xx	xxx	x	N.D.	N.D.
5. EC 3740	xxx	xxx	x	1810	****
6. N-4053	xx	x	--	2270	**
7. EH 1	--	xx	x	N.D.	N.D.
8. EH 2	xx	x	--	2200	**
9. EC 3890	xx	x	--	1120	****
10. EH 5667	xx	xxx	x	2010	***
11. EH 568	xxx	xxx	xx	2730	*
12. EH 5722	xx	x	x	2270	** 2/
13. EH 5665	xx	x	--	2665	*
14. EH 5678	xxx	xxx	xx	2410	*
15. EH 5731	xx	xxx	x	2270	**
16. EH 5669	x	x	--	2010	*
17. EH 5476	xx	xx	x	2070	***
18. EH Mix	xx	xxx	--	1320	****

-- No inhibition between 10-1000 ppm.
x At highest rate inhibition not less than 50%.
xx At highest rate inhibition to less than 50% for root and/or shoot.
xxx Complete suppression of growth at less than 1000 ppm.
xxxx Complete suppression of growth at 10 ppm.
*, **, ***, ****, Indication of comparative overall toxicity when scored on greenhouse results (See text).
2/ TCA, EH 5722 and EC 3740 applied at one-half the rate of other materials as preemergence; EC 3740 one-half the rate post emergence.
N.D. No data.

both instances on a common basis. Each chemical was rated on its relative toxicity as it was revealed in the germination test on each of the three species, and also on its overall toxicity as revealed in the greenhouse. These ratings are found in Table 43. For the laboratory test these ratings were; first, no inhibition indicated statistically; second, inhibition indicated but not inhibited at the highest rate to less than 50% (Tables 4, 5 and 6); third, inhibition established and either root or shoot, or both, reduced to less than 50% at 1000 ppm; fourth, complete suppression of development at concentrations between 10 and 1000 ppm; and fifth, complete suppression at 10 ppm.

In the greenhouse each species was given a rating on per cent of normal vigor for each treatment. Treatments were applied preemergence and post-emergence at two rates. The total of all the percentages for vigor assigned for the various treatments of one chemical was used as an indication of the overall toxicity of that compound as revealed in the greenhouse. Out of a possible score of 2800 the ratings ranged from 1120 to 2730. (Table 43 and Figures 8 to 11). On the basis of relative score, the chemicals were assigned to one of four groups; first, those which scored more than 2400 and which appeared to have no practical phytotoxic properties; second, those which scored between 2200 and 2400 and which showed little phytotoxicity; third, those which scored between 2000 and 2200 and which deserved further consideration; and fourth, those compounds which exhibited positive phytotoxic properties and scored less than 2000.

From the greenhouse results EH 5669, EH 5678, EH 568, and EH 5665, were placed in group one. EH 5665 and EH 5669 exhibited little general toxicity in the laboratory but both EH 568 and EH 5678 possessed phytotoxic properties not shown in the greenhouse. EH 5731, EH 2 and N-4053, were rated in the second group. EH 5722 was also placed in this group but preemergence treatments were made at one half the rates of the other chemicals. In this group no great amount of inhibition was exhibited by EH 5722, EH 2 and N-4055 in the laboratory, however, EH 5731 caused inhibition which was not reflected in the greenhouse. The third group included TCA, EH 5476, and EH 5667. Of these the latter showed itself to be more toxic than the other two in the germination test. TCA and EH 5476 showed less toxicity in the germination test than some of the materials found in groups one and two. IPC and EC 3740; two of the most toxic compounds in the laboratory test, were also classified as highly toxic in the greenhouse. EH Mix and EH 3890 both had potent growth inhibiting ability in the greenhouse which was not apparent in the germination screening test.

SUMMARY AND CONCLUSIONS

A review of published literature concerning the various tests now being used by commercial formulators and others interested in screening procedures indicate that none of these tests in use are completely satisfactory.

In this study eighteen chemical compounds were subjected to a test designed for the purpose of screening chemical compounds with herbicidal properties. The seeds of three plant species were incubated under blotters in petri dishes in a standard germinator for a period of 96 hours. The blotters were soaked in concentrations of 10, 100, 500 and 1000 ppm of the test herbicides, and a check was included in which case the blotters were soaked in distilled water. Mean root and shoot lengths were determined; these data were analyzed for variance associated with treatment and with regression in order to determine which chemicals possessed growth inhibitory properties under the conditions of the test.

To determine the reliability of this procedure many of the test chemicals were also evaluated in the greenhouse. Seven plant species were treated preemergence and post emergence at two rates. The treated plants were rated on their vigor which was expressed as a per cent of an untreated check. The total of these vigor ratings was used as an expression of the general toxicity of the chemical on growing plants.

The reactions of the roots and shoots of the three species to the 18 chemicals as exhibited in the laboratory test were compared graphically (Figures 1-5). There was a marked difference in the response of the seedlings to the various chemicals. While 2,4-D and IPC caused complete inhibition at 10 ppm, others, such as KH 5669, showed little inhibition even at the highest rate.

The reactions of the three species to specific herbicides varied widely. Wheat exhibited no inhibition to root or shoot length as a result of treatment with 7 of the 18 chemicals while the other two species showed no reaction to all rates of only one herbicide. Considering this phenomenon, it is evident that the validity of a screening test is enhanced by including more than one species. Species reaction differences found in the germination test with 2,4-D and TCA are also found in the field where the difference is of practical value.

Data of a number of tests indicated that regression was apparent and where sufficient data were available regression lines were determined. Since this test was merely a preliminary study, the range used for all chemicals was constant. As a result the range was not always adequate for the establishment of regression lines. In establishing the regression characteristics of herbicidal compounds it is suggested that the proper range of activity be determined before collecting data for regression coefficients and regression lines. A study of the regression lines reveals some outstanding differences in the reactions of the roots and shoots of individual species. While the regression lines tended to be very similar for some treatments others were markedly divergent. A regression coefficient of -22.8 was established for the shoot growth of Setaria when treated with TCA, whereas the coefficient for the root growth of Setaria with the same treatment was only -8.3. Alfalfa seedlings treated with EC 3890 had a regression coefficient of -19.6 for root growth but no inhibition was established for shoots. This lack of symmetry may be of considerable importance since both of these compounds possessed greater growth inhibiting qualities than their relative position in the germination test data would indicate.

By comparing the results of the germination test to those of the greenhouse test it was obvious that the former could not be used as the sole method for screening compounds. The response of highly active compounds like 2,4-D and its derivatives are quite evident in such a test. IPC, 2,4-D and EC 3740 were the most reactive in this test and obviously merit further testing. TCA, EC 3890 and EH Mix showed considerable promise in the greenhouse tests, but apart from the dissimilar behavior of roots and shoots of test species treated with TCA and EC 3890, the response to these chemicals appeared no more spectacular than that of many of the compounds of lesser toxicity as indicated in greenhouse tests. On the other hand, there were several compounds which exhibited relatively great inhibitory properties in the germination test, but which proved to be relatively inactive in the greenhouse.

Data of this investigation indicate that a seedling germination technique for the evaluation of unknown compounds of possible herbicidal properties may be highly useful but that it inadequately estimates possible field response of some materials. Subsequent greenhouse and field screening of these compounds with a wide range of plant species is essential for a valid estimation of their economic value as herbicides.

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